IN THE SPECIFICATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a divisional of United States Application No. 09/614,748, filed on July 12, 2000, now U.S. Patent 6,660,474, which is a continuation of International Application No. PCT/US99/00663, filed on January 12, 1999, which claims the benefit of United States Provisional Application No. 60/071,199, filed on January 12, 1998, and United States Provisional Application No. 60/098,279, filed on August 28, 1998, the disclosures of which are incorporated by reference herein.

[0005] It has been estimated that genetic factors account for 30-40% of blood pressure variability in humans (Ward, In *Hypertension: Pathophysiology, Diagnosis and Management*, Laragh JH. and Brenner BM eds., (Raven Press, Ltd., New York, NY), 81-100 (1990).) However, other estimates have suggested that genetic heritability of hypertension may be as high as 80% with 40% accounted for by one major gene (Cavalli, *et al.*, ~~In~~ in *The Genetics of Human Population*, (WH Freeman Co., South San Francisco, CA) 534-536 (1971)). The single major gene could effect blood pressure to such a significant extent that it would dominate many other genes that play a minor role in blood pressure control.

~~BRIEF DESCRIPTION OF THE DRAWINGS~~

[0017] Without being bound by any particular theory of operation, Applicants believe that a renal defect is responsible for a certain portion of hypertension in human subjects, and that the *GRK4* mutation either causes among other things, a direct or indirect ligand independent serine-hyperphosphorylation of the D1 receptor, resulting in its uncoupling from the G protein/effector complex. The result is that the natriuretic effect of dopamine is compromised and the

kidney is unable to properly balance sodium and water, leading to sodium retention and elevated blood pressure. More specifically, renal proximal tubules obtained from human hypertensive subjects, but not from normotensive subjects, demonstrate a defective coupling of the dopamine D1 receptor with adenylyl cyclase. The defective coupling is associated with a ligand-independent phosphorylation of the D1 receptor. Applicants have discovered at least six mutations in G protein related kinase type 4 (GRK4), that regulate ligand-independent phosphorylation of the D1 receptor in hypertensive patients.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] GRK4 is originally reported in Ambrose, *et al.*, Hum. Mol. Genet. *1*:697-703 (1993), and then more extensively characterized in Premont *et al.*, J. Biol. Chem. *271(11)*:6403-6410 (1996). *Premont* reports that GRK4 is highly abundant in testis only, *GRK4* mRNA being present to a small extent in brain and skeletal muscle. The *GRK4* gene, exclusive of promoter regions, spans approximately 75 kilobases (kDa), and is composed of 16 exons. The longest form of GRK4, with intact amino- and carboxyl-terminal alternative exon sequences, has been designated GRK4alpha. The deduced protein sequence contains 578 amino acids, with a predicted molecular mass of 66.5 kDa. The next shorter form, GRK4beta, lacks only the amino-terminal alternative exon, which is composed of codons, and thus contains 546 amino acids having a molecular mass of 62.kDa. GRK4 gamma is the isoform lacking only the carboxyl-terminal alternative exon, which is 46 codons. Thus, this isoform contains 532 amino acids, and has a predicted molecular mass of 61.2 kDa. GRK4gamma was formally called GRK4A. See Sallese *et al.*, Biochem. Biophys. Res. Commun. *199*:848-854 (1994). GRK4delta contains 500 amino acids with a predicted molecular mass of 57.6kDA, and is the shortest isoform. It lacks both alternative exons. GRK4delta was originally

designated IT11 and GRK4B. See Sallese *et al.*, *supra*, and Ambrose, *et al.*, *supra*. More recently, two additional isoforms have been discovered, namely: GRK4epsilon which lacks exons 13 and 15, contains ~~466~~ 486 amino acids with a predicted molecular mass of 53.6 kDa, and GRK4zeta which lacks exons 2, 13 and 15, contains ~~434~~ 454 amino acids with a predicted molecular mass of 49.9 kDa.

[0025] Five single nucleotide polymorphisms of *GRK4* are also known, namely: R65L (CGT to CTT); A142V (GCC to GTC); V247I (GTA to ATA); A486V (GCG to GTG) and D562G (GAC to GGC). See Premont, *et al.*, *supra*. Applicants have discovered that the R61L, the A142V and the A486V polymorphisms are associated with essential hypertension. Applicants have also discovered three additional polymorphisms prevalent in hypertensive individuals, namely: the double mutants R65L, A142V and R65L, A486V; and the triple mutant R65L, A142V, A486V. Table 1 shows the amino acid and corresponding nucleotide sequences of the six GRK4 isoforms. Amino acids and corresponding nucleotides that are changed in the polymorphs associated with essential hypertension are shown in bold. The sequences of the 5' untranslated regions of the epsilon and Zeta isoforms are not shown.

Table 1

MELENIVANS	LLLKARQGGY	GKKSGRSKKW	KEILTLPPVS	QCSELRHSIE	50	GRK4α
MELENIVANS	LLLKARQ----	----------	----------	----------		GRK4β
		----------	--------	------E		
MELENIVANS	LLLKARQGGY	GKKSGRSKKW	KEILTLPPVS	QCSELRHSIE		GRK4γ
MELENIVANS	LLLKARQ----	----------	----------	----------		GRK4δ
		----------	--------	------E		
MELENIVANS	LLLKARQGGY	GKKSGRSKKW	KEILTLPPVS	QCSELRHSIE		GRK4ε
MELENIVANS	LLLKARQ----	----------	----------	----------		GRK4ζ
		----------	--------	------E		
KDYSSLCDKQ	PIGR**R**LFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADDEDRSD	100	GRK4α
KDYSSLCDKQ	PIGR**R**LFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADDEDRSD		GRK4β
KDYSSLCDKQ	PIGR**R**LFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADDEDRSD		GRK4γ
KDYSSLCDKQ	PIGR**R**LFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADDEDRSD		GRK4δ
KDYSSLCDKQ	PIGR**R**LFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADDEDRSD		GRK4ε
KDYSSLCDKQ	PIGR**R**LFRQF	CDTKPTLKRH	IEFLDAVAEY	EVADDEDRSD		GRK4ζ
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	K**A**FEECTRVA	150	GRK4α
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	K**A**FEECTRVA		GRK4β
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	K**A**FEECTRVA		GRK4γ
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	K**A**FEECTRVA		GRK4δ
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	K**A**FEECTRVA		GRK4ε
CGLSILDRFF	NDKLAAPLPE	IPPDVVTECR	LGLKEENPSK	K**A**FEECTRVA		GRK4ζ
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTFRHY	RVLGKGGFGE	200	GRK4α
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTFRHY	RVLGKGGFGE		GRK4β
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTFRHY	RVLGKGGFGE		GRK4γ
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTFRHY	RVLGKGGFGE		GRK4δ
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTFRHY	RVLGKGGFGE		GRK4ε
HNYLRGEPFE	EYQESSYFSQ	FLQWKWLERQ	PVTKNTFRHY	RVLGKGGFGE		GRK4ζ
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL	250	GRK4α
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4β
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4γ
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4δ
VCACQVRATG	KMYACKKLQK	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4ε
VCACQVRATG	KMYACKKLQ	KRIKKRKGEA	MALNEKRILE	KVQSRFVVSL		GRK4ζ

AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL	300	GRK4α
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4β
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4γ
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4δ
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4ε
AYAYETKDAL	CLVLTIMNGG	DLKFHIYNLG	NPGFDEQRAV	FYAAELCCGL		GRK4ζ
EDLQRERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QRVRGRVGTV	350	GRK4α
EDLQRERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QRVRGRVGTV		GRK4β
EDLQRERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QRVRGRVGTV		GRK4γ
EDLQRERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QRVRGRVGTV		GRK4δ
EDLQRERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QRVRGRVGTV		GRK4ε
EDLQRERIVY	RDLKPENILL	DDRGHIRISD	LGLATEIPEG	QRVRGRVGTV		GRK4ζ
GYMAPEVVNN	EKYTFSPDWW	GLGCLIYEMI	QGHSPFKKYK	EKVKWEEVDQ	400	GRK4α
GYMAPEVVNN	EKYTFSPDWW	GLGCLIYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4β
GYMAPEVVNN	EKYTFSPDWW	GLGCLIYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4γ
GYMAPEVVNN	EKYTFSPDWW	GLGCLIYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4δ
GYMAPEVVNN	EKYTFSPDWW	GLGCLIYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4ε
GYMAPEVVNN	EKYTFSPDWW	GLGCLIYEMI	QGHSPFKKYK	EKVKWEEVDQ		GRK4ζ
RIKNDTEEYS	EKFSEDAKSI	CRMLLTKNPS	KRLGCRGEGA	AGVKQHPVFK	450	GRK4α
RIKNDTEEYS	EKFSEDAKSI	CRMLLTKNPS	KRLGCRGEGA	AGVKQHPVFK		GRK4β
RIKNDTEEYS	EKFSEDAKSI	CRMLLTKNPS	KRLGCRGEGA	AGVKQHPVFK		GRK4γ
RIKNDTEEYS	EKFSEDAKSI	CRMLLTKNPS	KRLGCRGEGA	AGVKQHPVFK		GRK4δ
RIKNDTEEYS	EKFSEDAKSI	CRM--------	----------	----------		GRK4ε
RIKNDTEEYS	EKFSEDAKSI	CRM--------	----------	----------		GRK4ζ
DINFRRLEAN	MLEPPFCPDP	HAVYCKDVLD	IEQFS**A**VKGI	YLDTADEDFY	500	GRK4α
DINFRRLEAN	MLEPPFCPDP	HAVYCKDVLD	IEQFS**A**VKGI	YLDTADEDFY		GRK4β
DINFRRLEAN	MLEPPFCPDP	HAVYCKDVLD	IEQFS**A**VKGI	YLDTADEDFY		GRK4γ
DINFRRLEAN	MLEPPFCPDP	HAVYCKDVLD	IEQFS**A**VKGI	YLDTADEDFY		GRK4δ
----------	----------P	HAVYCKDVLD	IEQFS**A**VKGI	YLDTADEDFY		GRK4ε
----------	----------P	HAVYCKDVLD	IEQFS**A**VKGI	YLDTADEDFY		GRK4ζ
ARFATGCVSI	PWQNEMIESG	CFKDINKSES	EEALPLDLDK	NIHTPVSRPN	550	GRK4α
ARFATGCVSI	PWQNEMIESG	CFKDINKSES	EEALPLDLDK	NIHTPVSRPN		GRK4β
ARFATGCVSI	PWQNE-----	----------	----------	----------		GRK4γ
ARFATGCVSI	PWQNE-----	----------	----------	----------		GRK4δ

ARFATGCVSI	PWQNE-----	---------	---------	----------	**GRK4ε**
ARFATGCVSI	PWQNE-----	---------	---------	----------	**GRK4ζ**

RGFFYRLFRR	GGCLTMVPSE	KEVEPKQC	578	GRK4α	(SEQ ID NO:1)
RGFFYRLFRR	GGCLTMVPSE	KEVEPKQC	546	GRK4β	(SEQ ID NO:2)
----------	-GCLTMVPSE	KEVEPKQC	532	GRK4γ	(SEQ ID NO:3)
----------	-GCLTMVPSE	KEVEPKQC	500	GRK4δ	(SEQ ID NO:4)
----------	-GCLTMVPSE	KEVEPKQC	~~466~~486	GRK4ε	(SEQ ID NO:5)
----------	-GCLTMVPSE	KEVEPKQC	~~434~~454	GRK4ζ	(SEQ ID NO:6)

Note: The bolded letters indicate the change in amino acid associated with hypertension R to L (argnine to leucine), A to V (alanine to valine), and A to V (alanine to valine).